# Supporting Information

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## SI Materials and Methods

**Recombinant Cell Culture.** FRT cells stably expressing human G551D CFTR (L. J. V. Galietta, Laboratorio di Genetica Molecolare, Istituto Giannina Gaslini, Genova, Italy) and NIH 3T3 mouse fibroblasts stably expressing human wild-type, human F508del CFTR (Michael J. Welsh, University of Iowa College of Medicine, Iowa City, IA) or the rat $\alpha$, $\beta$, and $\gamma$ subunits of ENaC (Mary Lang-Furr, University of North Carolina, Chapel Hill, NC) were cultured as previously described (1). All cells were maintained at 37 °C unless otherwise indicated, in HyQ CCM5 (HyClone, Logan, UT) with 1% heat inactivated FBS. For the transient transfection of mouse wild-type CFTR into FRT cells, a mouse CFTR expression vector was generated from mouse lung RNA and cloned into pcDNA3.1 Directional TOPO from Invitrogen (catalog no. 45–0158). FRT cells were grown in media containing Coons modified Ham's F12, 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin and were transiently transfected in suspension following the Lipo-Fectamine-2000.

**Isolation and Culture of HBE.** Whole lungs were provided by the National Disease Research Interchange (Philadelphia, PA) through an agreement with Cystic Fibrosis Foundation Therapeutics Inc. and were obtained from non-CF or CF individuals after autopsy or lung transplantation. After removal, the intact lung was packed in ice cold phosphate-buffered saline (PBS) and processed within 24 h. Frozen vials of dispersed G551D/F508del HBE were provided by Dr. Joseph M. Pilewski (University of Pittsburgh, PA). Non-CF and CF airway epithelia were isolated from bronchial tissue and cultured on 0.4 $\mu$m SnapWell™ culture inserts (Corning, catalog no. 3801) previously coated with NIH 3T3 conditioned media at a density of $5e^5$ cells/insert as previously described (2) with the following modifications: (*i*) Accutase (Innovative Cell Technologies Inc., San Diego, CA) was used to dissociate the cells; (*ii*) all plastic culture ware and the Costar Snapwell filters were precoated with NIH 3T3-conditioned media; and (*iii*) bovine brain extract (LONZA; Kit no. CC-4133, component no. CC-4092C) was added to the differentiation media. After 4 days, the apical media was removed and the cells were grown at an air–liquid interface for >14 days before use. This resulted in a monolayer of fully differentiated columnar cells that were ciliated.

**Ussing Chamber Recordings.** All cells were grown on Costar Snapwell cell culture inserts maintained at 37 °C before recording. The cell culture inserts were mounted into an Ussing chamber (VCC MC8; Physiologic Instruments, San Diego, CA) to record $I_T$ in the voltage-clamp mode ($V_{hold}$ = 0 mV) or the PD (PD = $V_{basolateral} - V_{apical}$) in the open-circuit recording mode. For FRT cells, the basolateral membrane was permeabilized with 360 $\mu$g/ml Nystatin and a basolateral to apical $Cl^-$ gradient was established. The basolateral bath solution contained (in mM); 135 NaCl, 1.2 $CaCl_2$, 1.2 $MgCl_2$, 2.4 $K_2HPO_4$, 0.6 $KHPO_4$, 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (Hepes), and 10 dextrose (titrated to pH 7.4 with NaOH). The apical NaCl was replaced by equimolar $Na^+$ gluconate (titrated to pH 7.4 with NaOH). For HBE cells, the $I_T$ was measured in the presence of a basolateral to apical $Cl^-$ gradient, whereas PD was measured in the presence of equimolar $Cl^-$ on the basolateral and apical sides. The normal-$Cl^-$ solution contained (in mM) the following: 145 NaCl, 3.3 $K_2HPO_4$, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, and 10 Hepes (pH adjusted to 7.35 with NaOH). The low-$Cl^-$ solution contained (in mM) the following: 145 Na-Gluconate, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 glucose, and 10 Hepes (pH adjusted to 7.35 with NaOH). All recordings were digitally acquired using Acquire and Analyze software (version 2; Physiologic Instruments, San Diego, CA).

**cAMP Measurements.** The total cAMP concentration (cellular and secreted) in recombinant FRT cells after test compound application was determined using a cAMP-Screen 96-Well Immunoassay System according the manufacturer's directions (Applied Biosystems, catalog no. T1502). Briefly, FRT cells were incubated for 15 min with test compound and then lysed and transferred to a 96-well assay plate provided with the kit. The plate was incubated at room temperature for 1 h, after which it was developed and luminescence emission was measured using the Acquest 384.1536 by LJL Biosystems. The cAMP concentrations were determined using a cAMP standard curve present in each plate.

**Patch-Clamp Recordings.** The single-channel activity of G551D CFTR, wild-type CFTR, and temperature-corrected F508del CFTR was measured using excised inside-out membrane patch recordings as previously described, using an Axopatch 200B (Axon Instruments Inc. ) patch-clamp amplifier (1). The pipette contained the following (in mM): 150 NMDG, 150 aspartic acid, 5 $CaCl_2$, 2 $MgCl_2$, and 10 Hepes (pH adjusted to 7.35 with Tris base). The bath contained (in mM) the following: 150 NMDG-Cl, 2 $MgCl_2$, 5 EGTA, 10 NaF, 10 TES, and 14 Tris base (pH adjusted to 7.35 with HCl). After excision, CFTR was activated by adding 1 mM Mg-ATP and 75 nM PKA (Promega Corp. , Madison, WI). The pipette potential was maintained at 80 mV. Because the $P_o$ for G551D CFTR is ≈1/20 of that observed for wild-type CFTR, it is difficult to accurately determine the number of channels in the membrane patch, which can lead to an overestimation of the actual $P_o$ for G551D CFTR. Because the trafficking and single-channel conductance of G551D CFTR is normal, the $P_o$ for G551D CFTR was corrected for the average number of wild-type CFTR channels observed in single-channel studies. The isolated $Na^+$ current in NIH 3T3 cells expressing ENaC was recorded using the whole-cell patch-clamp recording configuration. The pipette contained (in mM) the following: 150 CsCl, 1 $CaCl_2$, 2 $MgCl_2$, 5 EGTA, and 10 Hepes (pH adjusted to 7.35 with CsOH). The bath contained (in mM) the following: 150 NaCl, 2 $MgCl_2$, 5 EGTA, 10 TES, and 14 Tris base (pH adjusted to 7.35 with HCl). A voltage-ramp from a holding potential of +40 mV to −100 mV was applied every 20 s.

**Measurement of ASL Volume and CBF.** To monitor fluid absorption and CBF, G551D/F508del HBE were grown on 12-mm diameter inserts with 0.4-$\mu$m membrane pore size. The apical layer of the HBE cells was washed twice with absorption buffer (89 NaCl, 4 KCl, 1.2 $MgCl_2$, 1.2 $CaCl_2$, 10 Hepes, 16 Na-Gluconate, 10 glucose), followed by addition of 100 $\mu$l of absorption buffer to the apical layer. To prevent evaporation, the cell plates were wrapped in a damp paper towel and placed in a modular incubation chamber (Billups-Rothenberg Inc , catalog no. MIC-101) filled with a gas mixture of 5% $CO_2$ and 95% oxygen and maintained at 37 °C. To monitor the ASL volume, the fluid remaining after up to 72 h of incubation with test compounds was aspirated from the apical surface and placed in preweighed 1.5 ml Eppendorf tubes. The CBF was monitored as previously described (3). Briefly, cultured HBE were incubated with test

compound for 5 days and the cilia beating was recorded at 43 fps using a phase contrast AxioVert 200 microscope equipped with a 20X Zeiss Achroplan objective and a digital camera (Edmond Optics; EO-0413 Mono LE USB). Each frame was divided into six equal segments, in which three regions of interest (ROI), measuring $3 \times 3$ pixels, were placed over a single beating cilium. If no cilia beating were visible, the ROI was placed next to the cell body. The mean gray level was determined for each ROI using Image J (version 1.41o), and a Fourier transformation was used to measure the CBF.

**Statistical Analyses.** Statistical comparisons were made using ANOVA followed by Tukey's multiple comparison test or Student's $t$ test. Prism 5 (GraphPad Software, La Jolla, CA) was used for analyses. $P < 0.05$ was determined as significant. All data are presented as the mean $\pm$ SEM.


1. Van Goor F, et al. (2006) Rescue of DeltaF508 CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol* 290:L1117–L1130.
2. Galietta LJ, et al. (1998) An improved method to obtain highly differentiated monolayers of human bronchial epithelial cells. *In Vitro Cell Dev Biol Anim* 34:478–481.
3. Sisson JH, Stoner JA, Ammons BA, Wyatt TA (2003) All-digital image capture and whole-field analysis of ciliary beat frequency. *J Microsc* 211:103–111.

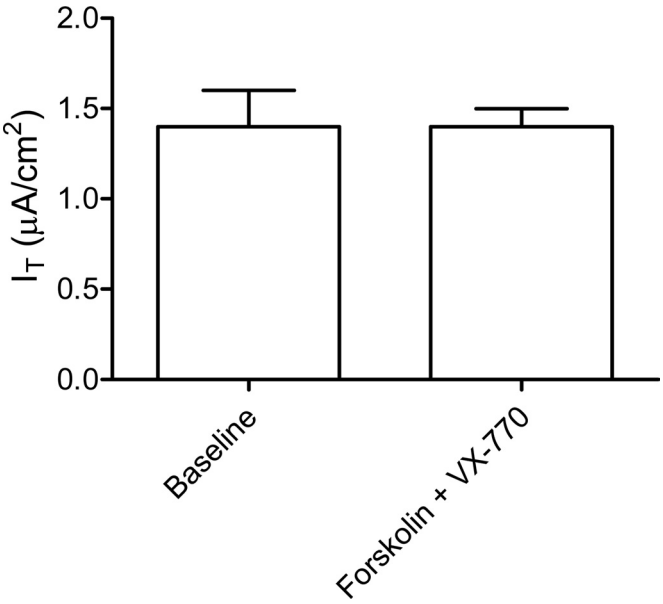


**Fig. S1.** Ussing chamber recording of the transepithelial current ($I_T$) before (baseline) and after addition of 10 $\mu$M forskolin and 10 $\mu$M VX-770 in basolateral membrane-permeabilized FRT cells that do not express CFTR.

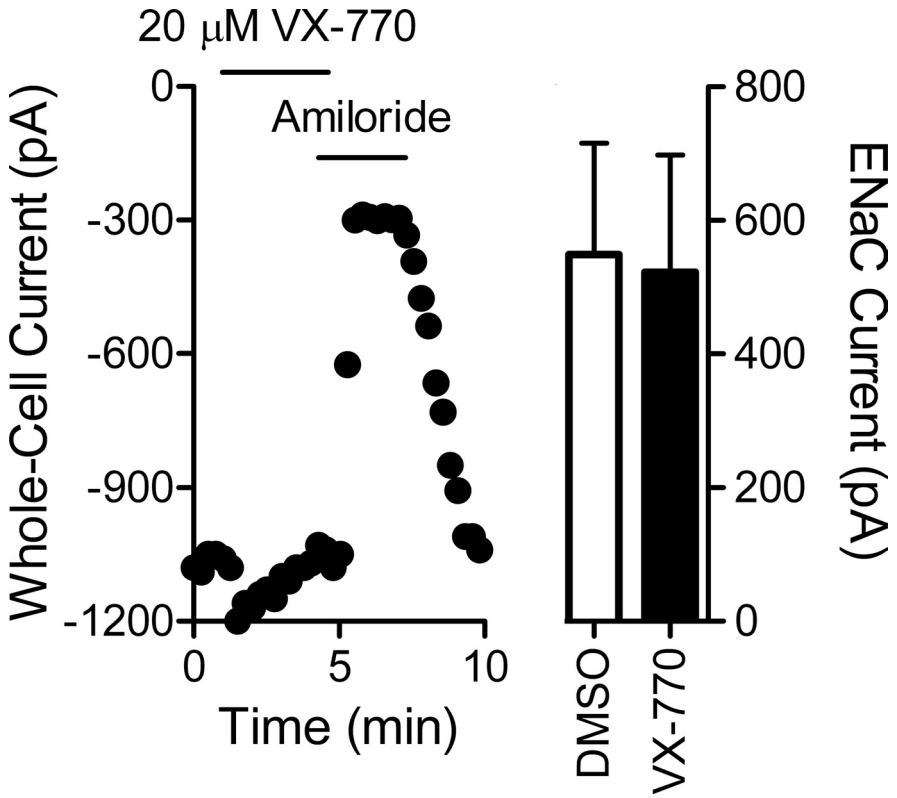


**Fig. S2.** *Left*, representative whole-cell recording of the $Na^+$ current in NIH 3T3 cells expressing ENaC; *Right*, amiloride-sensitive $Na^+$ current after DMSO or VX-770 addition ($n = 4$).

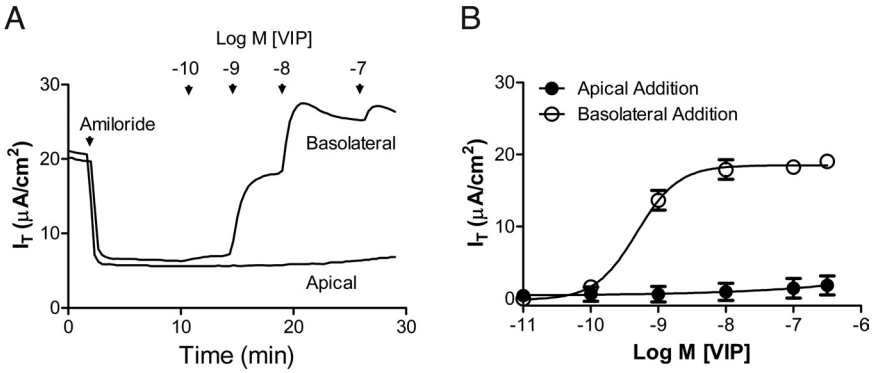


**Fig. S3.** Ussing chamber recording of the transepithelial current ($I_T$) response to increasing concentrations of vasoactive intestinal peptide (VIP) added to the mucosal (filled circles) or serosal (open circles) surface of cultured HBE isolated from the bronchi of an individual without cystic fibrosis. (*A*) Representative $I_T$ tracing of the response to amiloride (10 $\mu$M) followed by sequential addition of increasing concentrations of VIP to the serosal or mucosal surface. (*B*) Concentration response curve for VIP added to the mucosal (filled circles) or serosal (open circles) epithelial surface (mean $\pm$ SEM; $n = 3$).

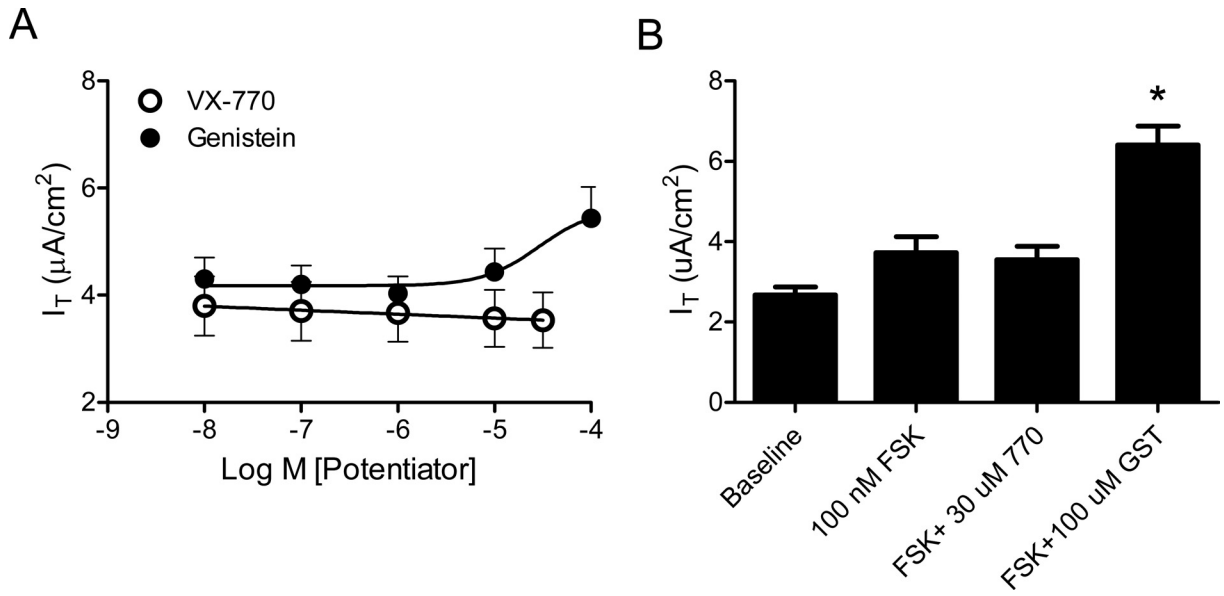


**Fig. S4.** VX-770 does not potentiate the forskolin (FSK)-stimulated transepithelial current ($I_T$) in Ussing chamber experiments using FRT cells expressing mouse wild-type CFTR. (*A*) The concentration response curve for VX-770 (open circles) and genistein (G, filled circles) in the presence of 100 nM FSK is shown for mouse wild-type CFTR expressed in FRT cells ($n = 3$). (*B*) The $I_T$ (mean ± SEM; $n = 4$) before FSK addition (baseline) and in the presence of 100 nM FSK alone or in combination with VX-770 (30 $\mu$M) or genistein (GST) (100 $\mu$M).